# Absolute sizing and label-free identification of nanoparticles by flow cytometry

- **Flow-SR relates ambiguous light scattering signals of flow cytometry to the diameter and refractive index of spherical single nanoparticles.**
- **Flow-SR enables label-free identification of nanoparticles.**
- **Flow-SR improves interpretation and comparison of flow cytometry data.**

Flow cytometry | Nanoparticles |

## Background

Rapid analysis of nanoparticles is essential to many disciplines. For example, biological nanoparticles in blood can be used as biomarker to recognize diseases at an early stage. In nanomedicine, nanoparticle characterization is a prerequisite for the development of drug-containing liposomes. In environmental science, the concentration of pollen, harmful cement dust, fly ash, metal nanoparticles, and nanoplastics require monitoring to limit damage to public health. In food production, label-free monitoring of the concentration of milk fat globules in cow milk or infant formula may improve quality control.

State-of-the-art flow cytometers are increasingly used to characterize single nanoparticles beyond kHz rate. Flow cytometers detect forward scattered light (FSC), side scattered light (SSC), and fluorescence of single nanoparticles. Because light scattering is a complex process, which depends on the size and refractive index (RI) of nanoparticles and the optical configuration of the flow cytometer, FSC and SSC are presented in arbitrary units. The arbitrary units, however, cause problems with data interpretation, data comparison and standardization. For example, from the scatter plot in Fig. 1A it is unclear what the size and composition of the measured particles is. Consequently, the scatter plots cannot be related to data generated by other analytical methods. Moreover, different flow cytometers provide entirely different scatter plots for the same sample, thereby impeding data comparison and multicenter (clinical) research. Thus, a tool to determine the physical properties of nanoparticles from flow cytometry data is urgently needed.

## The Invention

Flow cytometry scatter ratio (Flow-SR) is a new method to derive the diameter and RI of single nanoparticles from the FSC and SSC signals of a flow cytometer. Flow-SR is applicable to nanoparticles with a diameter ≤1.2 times the illumination wavelength. Direct access to the diameter in SI units solves the problems with data interpretation, data comparison and standardization.

## In vitro data

A flow cytometer (A50-Micro; Apogee, UK) was used to detect FSC and SSC of monodisperse reference beads with known diameter and RI (Fig. 1A). Using Flow-SR (Fig. 1B), we determined the diameter of all beads with ≤7% accuracy and ≤6% precision, which is more accurate and precise than techniques dedicated to size nanoparticles in suspension, such as nanoparticle tracking analysis and resistive pulse sensing. Also the determined RI of all beads is within the expected range. Furthermore, we demonstrated RI-based differentiation of particles from polydisperse oil emulsions having RI 1.36, 1.40 and 1.46. Furthermore, we used Flow-SR to demonstrate RI-based, label-free differentiation between extracellular vesicles (EVs) and lipoprotein particles in blood plasma samples and between EVs and milk fat globules in cow milk.

## Application / opportunity

Because Flow-SR is relatively easy to implement, widely applicable, and more accurate and faster than existing techniques to size nanoparticles in suspension, we expect immediate applications. Main applications are standardization, data comparison, data

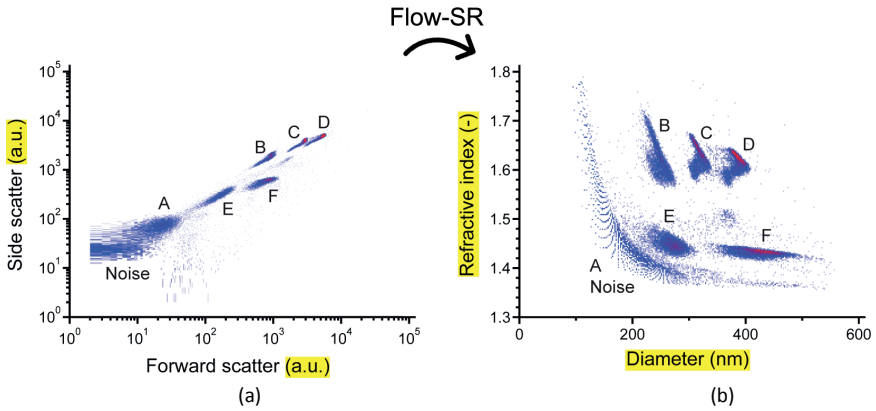


Fig. 1 (a) Side scatter versus forward scatter of nanoparticles measured by flow cytometry. Because the signals are expressed in arbitrary units (a.u.), the physical properties of the nanoparticles remain unknown. (b) After application of Flow-SR, the diameter in nanometers (nm) and the refractive

interpretation, and label-free differentiation between nanoparticles. Specific application include rapid label-free identification and sizing of EVs, lipoprotein particles, viruses, bacteria, nanoplastics, milk fat globules, reference materials, liposomes, pollen, cement dust, fly ash, and metal nanoparticles.

## R&D status/data

Flow-SR has been implemented foron an Apogee A60-Micro, Apogee A50-Micro and BD Influx flow cytometer. Other flow cytometers are suitable if they have nanoparticle sensitivity on the FSC and SSC detector.

## What are we looking for?

Partner who is willing to license and further develop the technology, preferably in collaboration with AMC.

## Intellectual Property

The initial patent application covering the technology was filed in September 2015, followed by a PCT application in 2016. The decision to enter the national phase is due in March 2018.

## Inventors

- Dr. Edwin van der Pol, Biomedical Engineering & Physics and Laboratory of Experimental Clinical Chemistry, Academic Medical Center

Edwin is Postdoc in the department of Biomedical Engineering & Physics and works on the detection of EVs as clinical biomarker. Edwin focuses on modelling light scattering of nanoparticles, automating flow cytometry data analyses, standardization and quality control.

- Dr. Frank Coumans, Biomedical Engineering & Physics and Laboratory of Experimental Clinical Chemistry, Academic Medical Center

Frank is Postdoc in the department of Biomedical Engineering & Physics and coordinates the CANCER-ID consortium, which aims for using EVs as biomarker for cancer. Frank focuses on hardware modifications of nanoparticle flow cytometers and standardization.

- Dr. Rienk Nieuwland, Experimental Clinical Chemistry, Academic Medical Center

Rienk is group leader of the Laboratory Experimental Clinical Chemistry and develops clinical biomarkers based on EVs.

- Prof.Dr. Ton van Leeuwen, Biomedical Engineering & Physics, Academic Medical Center

Ton is group leader of the department of Biomedical Engineering & Physics and bridges the gap between engineering and physics at one side and life sciences and clinical medicine at the other side. Ton has a specific focus on biomedical optics.

## Key publications

E. van der Pol, L. de Rond, F.A.W. Coumans, E.L. Gool, A.N. Böing, A. Sturk, R. Nieuwland, and T.G. van Leeuwen. Absolute sizing and label-free identification of extracellular vesicles by flow cytometry. Nanomedicine (2018).